CURRENT STATUS
REVIEW

Human gastrointestinal nematode infections: are new control methods required?

Gillian Stepek*[1], David J. Buttle[†], Ian R. Duce* and Jerzy M. Behnke*

*School of Biology, University of Nottingham, Nottingham, UK; and [†]Division of Genomic Medicine, University of Sheffield, Sheffield, UK
[1]Current address: Department of Veterinary Parasitology, Faculty of Veterinary Medicine, University of Glasgow, Glasgow, UK

Summary

Gastrointestinal (GI) nematode infections affect 50% of the human population worldwide, and cause great morbidity as well as hundreds of thousands of deaths. Despite modern medical practices, the proportion of the population infected with GI nematodes is not falling. This is due to a number of factors, the most important being the lack of good healthcare, sanitation and health education in many developing countries. A relatively new problem is the development of resistance to the small number of drugs available to treat GI nematode infections. Here we review the most important parasitic GI nematodes and the methods available to control them. In addition, we discuss the current status of new anthelmintic treatments, particularly the plant cysteine proteinases from various sources of latex-bearing plants and fruits.

Keywords

anthelmintic, control, gastrointestinal nematodes, human, plant cysteine proteinases, resistance

Parasitic infections are a major medical problem throughout the world, especially in developing countries where they cause more morbidity and mortality than other infectious diseases and are the primary cause of death. There are two main groups of parasites: (a) the protozoa, which are unicellular organisms and include the malaria parasite, *Plasmodium*; and (b) the helminths, which are metazoan organisms and include the cestodes, trematodes and nematodes. The protozoa are responsible for the majority of the mortality associated with parasitic infections, while the helminths generally produce long-term (or chronic), debilitating diseases: one of the reasons why there is more public awareness of parasitic protozoan infections than of infections with helminths. In this paper, we review the major human gastrointestinal nematodes, the problems associated with the control of these parasites and a potential new therapeutic approach.

Gastrointestinal nematode infections of humans

Gastrointestinal (GI) nematode (soil-transmitted helminth) infections are amongst the most prevalent worldwide, although this is largely acknowledged only by those working in this field. It is estimated that there are 3.5 billion cases worldwide, of which 450 million are individuals who are seriously ill as a result, the majority of who are children, and of which 44 million are pregnant women infected with hookworms. Approximately 125 000 deaths occur per year, and these are mainly due to infections with the hookworms, *Ancylostoma duodenale* and *Necator americanus*, or the roundworm, *Ascaris lumbricoides*. There are 300–500 million cases of malaria per year and, although this number is much less than that for GI nematode infections, the number of deaths attributable to malaria is far greater, reaching 3 million per year (http://www.who.int/wormcontrol/en/; http://www.library.csi.cuny.edu/~davis/faculty_page/Parasit_links/parasitology_links.html). According to Chan (1997), the prevalence of GI nematode infections has remained unchanged in over 50 years, with 39 million disability-adjusted life years (DALYs) lost due to these parasites when compared with 35.7 million lost to malaria or 34.1 million lost to measles. How can this be the case with modern medical practices? The majority of infections with GI nematodes remain asymptomatic, and those cases which do cause morbidity are not directly fatal, in contrast to malaria. This may be one reason why GI nematode infections have been neglected diseases in terms of public recognition and research funding.

Of the 342 helminth species that infect humans (Crompton 1999), the species of greatest medical importance are *Ascaris lumbricoides* (roundworm), *Ancylostoma duodenale* and *Necator americanus* (hookworms), *Trichuris trichiura* (whipworm), *Enterobius vermicularis* (pinworm) and *Strongyloides stercoralis* (threadworm) (Table 1). For more than 50 years, the number of cases of GI nematode infections has increased with the global population, such that over 50% of the world's population are affected by the six major GI nematode species (Chan 1997; Horton 2003). The majority of these people live in the developing countries, with those living in rural and urban slums most at risk. This is probably due to the poor housing, overcrowded living conditions, lack of adequate sanitation and hygiene and poor education and health care in these areas, all of which are associated with poverty. In the case of *S. stercoralis* in urban slums, one risk factor for transmission of infection is believed to be close contact between individuals, linked with poor personal hygiene (Conway *et al.* 1995). In addition, warm and humid climatic conditions appear to favour the survival of free-living larval stages of species such as *N. americanus*, *A. duodenale* and *S. stercoralis* (Kappus *et al.* 1994), with the latter parasite endemic in tropical and sub-tropical countries, including South East America, the Far East, West Africa, Italy and Australia. Although GI nematodes are largely restricted to the tropics and sub-tropics, infections can occur in the Northern hemisphere when ambient conditions are favourable. For example, hookworms caused anaemia in tin miners in Cornwall at the start of the 20th century and also caused problems among engineers in the Alps (Boycott & Haldane 1903; Boycott 1911; Foster 1965). In both cases, the poor hygiene standards of the workers, in warm, moist conditions, favoured transmission. In general, however, infections with GI nematodes, with the exception of *E. vermicularis*, are less frequent in temperate climates and technologically developed societies, such as the USA and Western Europe; nevertheless, a proportion of these populations still become infected, despite the higher standards of hygiene and sanitation (Kappus *et al.* 1994). In particular, *E. vermicularis* infections, whilst being present throughout the tropics, are also widespread in the Northern hemisphere, such as in the UK, unlike infections with the other major GI nematodes; this is possibly due to the low temperatures and high

Table 1 The major gastrointestinal (GI) nematode parasites of humans

GI nematode (species name)	GI nematode (group name)	Number infected	Distribution	Transmission
Ancylostoma duodenale/ Necator americanus	Hookworm	1.3 billion	Worldwide, especially tropical regions	Skin contact with contaminated soil
Ascaris lumbricoides	Roundworm	1.3 billion	Worldwide, especially tropical regions	Ingestion of eggs
Trichuris trichiura	Whipworm	1.05 billion	Worldwide, especially tropical regions	Ingestion of eggs
Enterobius vermicularis	Pinworm	209 million	Worldwide	Ingestion of eggs; occasionally inhaled
Strongyloides stercoralis	Threadworm	30 million	Worldwide, especially tropical regions	Skin contact with contaminated soil; autoinfection

humidities which favour egg development. Infections with this nematode are the least harmful, and are considered more of a nuisance than a serious disease. Nevertheless, they are most common in crowded residences, particularly in school children 5–10 years old, and are spread easily between all family members, with frequent reinfection (Gonzalez & Javier de la Cabada 1987; Cook 1994; Kucik *et al.* 2004).

Transmission and life-cycle of human GI nematodes

The six GI nematode species of major importance, *A. duodenale*, *N. americanus*, *A. lumbricoides*, *T. trichiura*, *E. vermicularis* and *S. stercoralis*, have direct life-cycles, i.e. only one host is involved. These six nematode species are all highly specific to humans, with no animal reservoirs of infection for any species. Although some animal species, such as pigs, can become infected with the human GI nematodes, the life-cycle cannot reach completion in these foreign hosts. The eggs or larvae of all the major nematodes, with the exception of *E. vermicularis*, require a period of development in the soil to become infective before transmission to the human host. This requirement, combined with a similar geographical distribution, generates a high frequency of concurrent multiple species infections (usually *A. lumbricoides* and *T. trichiura*; Booth & Bundy 1992), especially in areas where several species are sympatric. In these endemic regions, multiple worm infections are more common than infections with a single species, but are still largely asymptomatic when the worm burden is low. However, multiple infections can exacerbate the pathology (Booth *et al.* 1998). Infections with *A. lumbricoides* and *T. trichiura* are more likely to be transmitted within the domestic situation where eggs may persist in household dust, whereas hookworm infections are more often transmitted in the field, where shoes are worn infrequently. Whereas *Ascaris* and *Trichuris* can only infect via oral ingestion, hookworms can also infect the host by skin penetration (see below). This is why the wearing of shoes is a major factor in the prevention of hookworm transmission (Killewo *et al.* 1991).

The life-cycles of the major GI nematodes of humans are essentially similar (Figure 1), but they do have specific differences. In all, the adult worms reproduce sexually and the mature female worms produce and release eggs into their immediate environment of the human intestine. In most species, these eggs pass into the external environment via host faeces and then the L1 (first larval stage) develop within the eggs. The nematode species differ with respect to subsequent development, although in all cases, the L1 develop through four further stages (L2, L3, L4 and pre-adults), with each stage preceded by a cuticular moult, and each moult causing

an increase in parasite size. However, there are important differences between species as to where and when these moults occur. Finally, the pre-adult worms become mature adult worms, but both the site within the host and the time for maturation of the pre-adult stage to fully fecund (egg-laying) females differ between nematode species.

The eggs of both *A. lumbricoides* and *T. trichiura* require a period of external development to become infective even though the eggs do not hatch in the external environment. The L1 of *T. trichiura* develop in the eggs and it is this stage which is infective to humans. For further development to occur, the L1 have to be ingested, and this usually occurs via contaminated food or water. On ingestion, the eggs (containing the infective L1) hatch in the small intestine, from where the L1 migrate to the colon of the large intestine and develop, through four moults, to mature adult worms. For *A. lumbricoides*, the L1 develop and undergo two moults to the infective L3 within the eggs, whilst in the external environment. As for *T. trichiura*, the eggs containing the infective L3 enter the human host by ingestion, where they hatch and release the L3 into the duodenum. This is followed by tissue migration to the lungs, via the liver, where the third moult to L4 occurs. The L4 enter the trachea and are then swallowed and enter the intestine, where final maturation to the adult worms occurs (Whitfield 1993; Wakelin 1996a,b).

In contrast, the eggs of hookworms (*A. duodenale* and *N. americanus*) hatch externally and release the L1, which develop as free-living stages through two moults to the infective L3. Infection of humans by these L3 is largely by active skin penetration. The L3 migrate to the lungs and the trachea, from where they are swallowed, and enter the small intestine to complete their development through the two remaining moults and final maturation to adult worms. Whereas *N. americanus* can only infect humans via skin penetration, *A. duodenale* can infect both by skin penetration and by ingestion of the infective L3. Following ingestion, these L3 migrate directly to the small intestine where they develop, through two moults, to mature adult worms (Whitfield 1993; Wakelin 1996a,b).

Strongyloides stercoralis is the exception in that the parasitic stages are parthenogenic female worms, which release eggs that hatch internally in the host's intestine, releasing the L1. The L1 usually pass out of the host via faeces, but in some cases, their development can be accelerated whilst still in the host. In such cases, the L1 moult twice to the infective L3, which penetrate the gut or the perianal skin surface and migrate to the lungs (autoinfection). More commonly, the L1 are released in the host faeces and then follow either the free-living or parasitic developmental cycle. In the parasitic cycle, the L1 develop through two moults to the infective
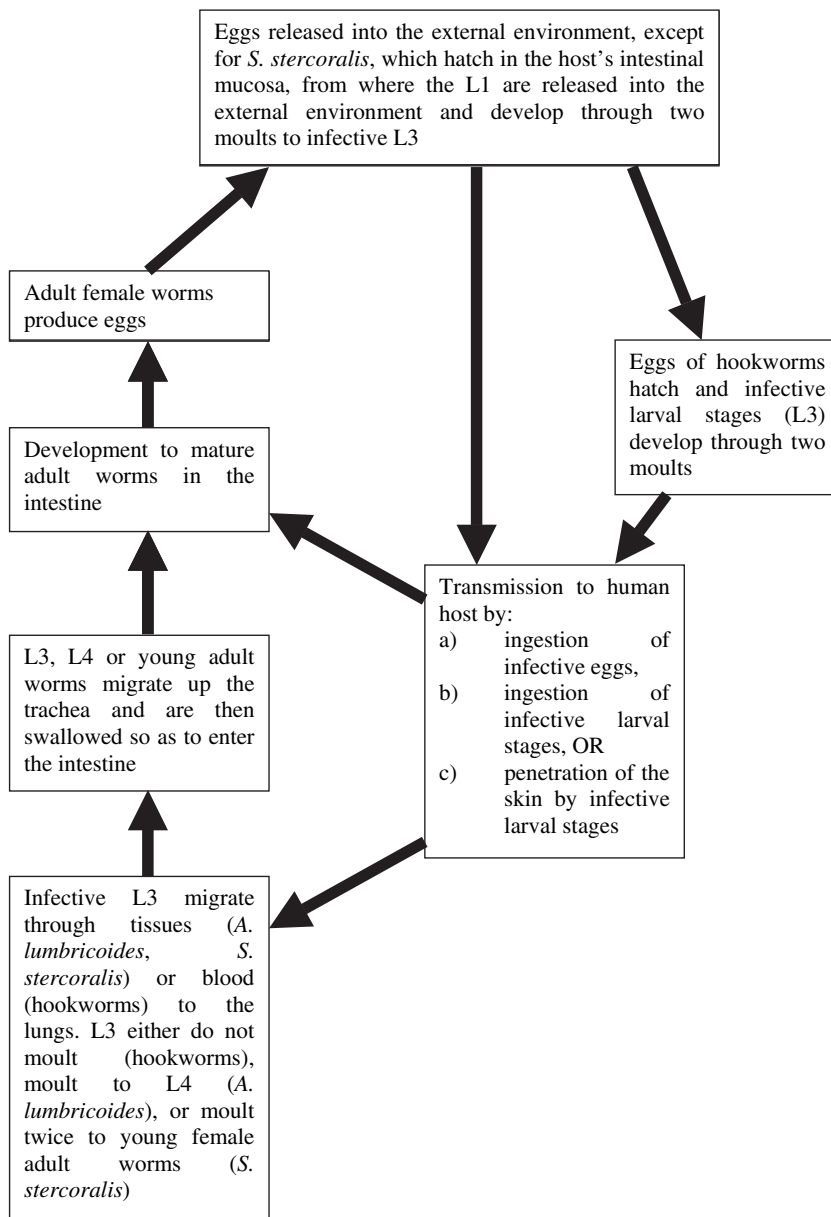
Eggs released into the external environment, except for *S. stercoralis*, which hatch in the host's intestinal mucosa, from where the L1 are released into the external environment and develop through two moults to infective L3

Adult female worms produce eggs

Development to mature adult worms in the intestine

Eggs of hookworms hatch and infective larval stages (L3) develop through two moults

L3, L4 or young adult worms migrate up the trachea and are then swallowed so as to enter the intestine

Transmission to human host by:
a) ingestion of infective eggs,
b) ingestion of infective larval stages, OR
c) penetration of the skin by infective larval stages

Infective L3 migrate through tissues (*A. lumbricoides*, *S. stercoralis*) or blood (hookworms) to the lungs. L3 either do not moult (hookworms), moult to L4 (*A. lumbricoides*), or moult twice to young female adult worms (*S. stercoralis*)

Figure 1 The life-cycle of human gastrointestinal (GI) nematodes, with the exception of *Enterobius vermicularis*.

L3, where no further development can occur until contact with a relevant host is made. In contrast, the L1 undergo the two moults to the L3 and then continue their development to the L4 and finally to the free-living adult worms during the free-living cycle, which is the only situation where male adult worms of *S. stercoralis* exist. In this latter route of development, the nematode can cycle through several generations as free-living organisms before reverting to the parasitic form. However, this complete free-living cycle can only occur when environmental conditions are favourable. Under unfavourable conditions, the L1 develop into infective L3, which can only undergo further development as part of the

parasitic life-cycle in the human host. Whether generated through the free-living or parasitic cycles, the infective L3 penetrate the skin and migrate to the lungs where they moult twice to become young parthenogenic female worms. These young adult worms migrate to the small intestine, via the trachea, and release their eggs. Therefore, not only does *S. stercoralis* infect humans by skin penetration, it is the only GI nematode that can undergo autoinfection of the human host, causing chronic infections lasting for as long as 40 years (Whitfield 1993; Wakelin 1996a,b).

The life-cycle of *E. vermicularis* differs from that of the other major human GI nematodes in that the L1 develop in

the eggs on the perianal skin or under the fingernails. The majority of the infective eggs are ingested, or inhaled and swallowed after being coughed up, and hatch in the small intestine, where they release the L1. These L1 undergo four moults to the adult stage, usually in the large intestine and the appendix. Gravid females then migrate to the anus and deposit eggs on the perianal skin (Whitfield 1993; Wakelin 1996a,b).

The survival of nematodes in the GI tract is favoured by the high availability of food, and an easy exit to the external environment ensures continuation of the life-cycle. Whereas most of the major GI nematodes obtain their nutrients by attaching and feeding on the mucosa of the intestinal tract, *Ascaris* also feeds on the contents of the intestinal lumen. Hookworms attach to the villi of the gut, abrading the mucosal surface and feeding on the mucosal tissues of the intestine, internalizing boluses of tissue and sucking in blood from the underlying capillaries, making anaemia a prominent symptom of the disease caused by these parasites (Wakelin 1996b).

Zoonoses

Zoonotic diseases are of worldwide importance, and involve a large range of animals that are consumed by humans or associated with them. However, it is not possible to cover them all in this review, and so we have concentrated on the nematodes that are acquired from household pets, predominantly dogs. Zoonoses are particularly prevalent in rural areas of developing countries, such as India. Here, animals are often found living alongside humans in conditions of overcrowding, poor socio-economy and poor sanitation and hygiene, and there is frequently insufficient medical care and veterinary services and an unawareness of zoonotic diseases.

One of the most important zoonotic infections caused by GI nematodes is toxocariasis, caused by the dog parasite, *Toxocara canis*, which is closely related to *A. lumbricoides* and has a similar life-cycle. This nematode has a worldwide distribution, and is one of the most common zoonotic GI nematode infections in children. Due to environmental contamination by *Toxocara* eggs in areas frequented by children, such as sand-pits, frequent transmission of the nematode occurs from soil contaminated with dog faeces to humans (Schantz 1991; Traub *et al.* 2005). The majority of people infected with *Toxocara* remain asymptomatic, whilst a minority develop the severe or fatal diseases of visceral larva migrans, ocular larva migrans and covert toxocariasis. In these diseases, the eggs hatch in the small intestine and the larvae migrate to the liver, lungs, eyes and other body organs, where they cause tissue necrosis, chronic liver disease, oedema, haemorrhage and eosinophilia. Most cases are preventable by improvements to hygiene, elimination of the parasite from dogs (especially puppies, due to transplacental transmission), and preventing children from playing in areas frequented by infected animals (Hartleb & Januszewski 2001; Baboolal & Rawlins 2002; Traub *et al.* 2005).

Infection of humans with the canine hookworm, *Ancylostoma ceylanicum*, occurs in areas of low socio-economic conditions, such as the Philippines and South America, where proper footwear is unaffordable and infected dogs reside in the same environment as humans (Velasquez & Cabrera 1968). However, it is not just the poorest regions of the world where canine hookworms can infect humans: *Ancylostoma caninum* frequently infects humans in tropical and temperate countries, including Australia and the USA. This is mainly due to the large number of dogs owned as pets and to the behaviour of both humans and dogs, e.g. people tend to walk barefoot on damp grass or on the beach, where they may be exposed to the infective skin-penetrating larvae as these areas are also frequented by infected dogs. Infections with *A. caninum* can result in eosinophilic enteritis, which is mainly restricted to the small intestine. Although eosinophilic enteritis is treatable, it can be recurrent, possibly following a seasonal pattern as in dogs, and this is believed to be due to the reactivation of dormant L3. The presence of the infective L3 of canine hookworms in humans can also lead to the development of cutaneous larva migrans in these abnormal hosts (Prociv & Croese 1996; Traub *et al.* 2005).

Consequences of infections with GI nematodes

In the case of heavy infections with GI nematodes, the most common complaints are intestinal, such as diarrhoea, abdominal pain and, in the case of *A. lumbricoides*, obstruction of the gut (Gilles 1968; Gonzalez & Javier de la Cabada 1987; Clinch & Stephens 2000; Kucik *et al.* 2004). However, these parasitic infections have more serious consequences, as outlined below.

Anaemia

Anaemia is a major consequence of infection with GI nematodes, in particular the hookworms, but heavy *T. trichiura* infections have also been found to cause iron-deficiency anaemia (Gilgen *et al.* 2001). The majority of cases of hookworm-induced anaemia occur in people who live rurally in developing countries and rely on agricultural labour for the main family income. Anaemia reduces the physical ability to carry out the work associated with this way of life, leading

to poor nutrition and increased hookworm infection, thus setting up a vicious circle (Crompton 1986). The extent of anaemia is dependent on the intensity of infection, the infecting nematode species (*A. duodenale* causes much greater blood loss than *N. americanus*), and the level of iron intake and host nutritional status (Albonico *et al.* 1998).

The severity of iron-deficiency anaemia is greater in pregnant women infected with hookworms, compared with non-pregnant infected women, due to the natural increased iron requirement during pregnancy. Increasing severity of anaemia can lead to the death of the woman and increased risks to the unborn foetus, such as premature delivery. Thus, hookworms are a major contributing factor to the worldwide incidence of anaemia, particularly in malnourished pregnant women in the developing countries (Bundy *et al.* 1995; Dreyfuss *et al.* 2000).

Malnutrition

Livestock infected with GI nematodes vary in their ability to cope with these infections, and this ability, together with the severity of infection, is dependent on the nutritional status of the host (Coop & Kyriazakis 2001). It is believed that the same is true for GI nematode infections of humans. Malnutrition occurs in people living in conditions where GI nematode infections are endemic, i.e. high levels of poverty, poor sanitation and a lack of adequate hygiene standards, and results in iron deficiency, resulting in anaemia (see above). Infections with GI nematodes are more problematic in children suffering from malnutrition (Stephenson *et al.* 2000). In the young, not only do intestinal nematodes have a detrimental effect on the host's nutritional status, but they also cause a loss of appetite and affect the host's physical, cognitive and social development (Crompton 1986; Dossa *et al.* 2001). These latter effects are largely due to the malnourished state of the helminth-infected children (Levav *et al.* 1995). Malnutrition is exacerbated after infection with GI nematodes because the nematodes damage the intestinal mucosal epithelial cells whilst feeding, resulting in the prevention of nutrient absorption by the host and leading to stunted growth (Stephensen 1999). This, in turn, has consequences for the work productivity of adults in developing countries (Guyatt 2000). A single dose of albendazole to helminth-infected children in Kenya greatly improved their growth rate, weight, physical fitness and activity and appetite (Stephenson *et al.* 1993). Thus, by treating GI nematode infections, normal growth rate and appetite are restored, and productivity is also increased, especially in developing countries where the work is physically demanding.

School attendance and cognitive function

Moderate to heavy burdens of *T. trichiura* or hookworms in school children result in poor school attendance and low cognitive function when compared with the performance of uninfected children. The effect on cognitive function stems from malnutrition (see above), with or without anaemia, and, in undernourished children, is related to the intensity of GI nematode infection. However, the poor cognitive performance is reversible following treatment with anthelmintic drugs. Therefore, improvements in nutrition and lowering the incidence of infection are likely to lead to improvements in school performance (Nokes *et al.* 1992a,b; Simeon *et al.* 1995).

Secondary infections

There are two forms of severe infection with *S. stercoralis*: hyperinfective strongyloidiasis and disseminated strongyloidiasis, both of which can be life-threatening in the more susceptible immunosuppressed patients. Both severe forms involve the presence of a massive worm burden. These worms are concentrated in the intestinal and respiratory tracts in the hyperinfective form, but are spread throughout many organs, including the central nervous system, during disseminated strongyloidiasis. Both severe forms cause gastrointestinal, cutaneous and respiratory problems and, in the case of the disseminated disease, these are often fatal due to the increased risk of meningitis and other secondary bacterial infections resulting from infection with this nematode (Gilles 1968; Bwibo 1971; Milder *et al.* 1981; Gonzalez & Javier de la Cabada 1987; Stürchler 1987; Fisher *et al.* 1993; Jain *et al.* 1994; Schneider & Rogers 1997; Tsai *et al.* 2002; Keiser & Nutman 2004).

Infections with the major human GI nematodes impair the immune response to other serious infections, such as tuberculosis (TB) and human immunodeficiency virus (HIV), which are each controlled by a Th1 immune response. This type of immune response is downregulated by helminth infections; hence, the potential contribution to the rising numbers of TB and HIV infections in the developing countries. Helminth infections also induce the production of the T regulatory cytokines, interleukin (IL)-10 and transforming growth factor (TGF)-β, which are immunosuppressive and are believed to inhibit immune responses that protect against TB. This downregulation of the host's immune response is believed to be responsible for the more rapid progression of HIV infection to AIDS in the developing countries (Bentwich *et al.* 1999; Othieno *et al.* 1999; Elliott *et al.* 2003). Individuals with helminth infections show marked Th2 immune

responses and are chronically immune-activated, which increases their susceptibility to HIV and TB. The vaccines under current development against HIV and TB may have no effect in areas where helminth infections are endemic, i.e. the areas of highest HIV and TB incidence. This is due to the pre-existing Th2 immune responses of the infected individuals who may not be able to develop a protective Th1 response upon vaccination (Bentwich *et al.* 1995; Borkow & Bentwich 2000). However, one potential vaccine candidate against HIV involves the use of an adjuvant which changes the immune response of affected individuals from Th2 to Th1. Adjuvants that do not cause a change to a Th1 response have no effect on the protection against these infections (Ayash-Rashkovsky *et al.* 2002). Thus, the existing presence of helminth infections increases the susceptibility of individuals to secondary infections requiring a Th1 immune response for protection.

Control of GI nematodes of humans

Anthelmintics

Now, the major means of controlling human GI nematode infections is by the administration of one of the four chemotherapeutic anthelmintic drugs recommended by the WHO for the treatment of these infections. These drugs are albendazole, mebendazole, levamisole and pyrantel, and there is at least one anthelmintic drug which can be used to treat each of the major GI nematodes of humans (Table 2). These drugs belong to two distinct classes: group 1, the benzimidazoles (albendazole and mebendazole), and group 2, the imidazothiazoles/tetrahydropyrimidines (levamisole

and pyrantel). There is a third class of anthelmintics, the macrocyclic lactones (group 3; e.g. ivermectin), which are used in the treatment of GI nematode infections of livestock, but which have only recently been registered for use in humans against strongyloidiasis in France, Australia and the USA, although ivermectin has been available for use against filarial nematodes for several years (Albonico *et al.* 1999).

The benzimidazoles are broad spectrum drugs that bind to free β-tubulin, inhibiting its polymerization and so interfering with microtubule-dependent glucose uptake by the parasite. The imidazothiazoles/tetrahydropyrimidines stimulate the nicotinic acetylcholine receptors, resulting in over-stimulation, blockade of the neuromuscular junctions, and rigid paralysis of the worms. The parasites are then unable to move in the intestinal tract and are swept out by the peristaltic action in the intestine. The macrocyclic lactones act by opening glutamate-gated chloride channels, increasing chloride ion conductance, leading to defects in neurotransmission and flaccid paralysis. There is a fourth class of anthelmintics, the heterocyclic ethyleneamines, of which the best known member, piperazine, is only used against *A. lumbricoides* and *E. vermicularis*. This drug acts by reversibly inhibiting neuromuscular transmission by stimulating gamma-aminobutyric acid (GABA) receptors in nematode muscle, causing flaccid paralysis of the worms, which are then removed by normal intestinal peristalsis (Rang *et al.* 2003).

These anthelmintics are relatively safe, having a very few minor gastrointestinal side-effects. However, none are recommended for use by pregnant women, particularly those in the first trimester. Although they can be administered in single doses, these drugs are more effective during treatment with

Table 2 Percentage cure rates for the anthelmintics currently used to control infections with human gastrointestinal (GI) nematodes

Anthelmintic	GI nematodes (%)				
	Hookworms	*A. lumbricoides*	*T. trichiura*	*E. vermicularis*	*S. stercoralis*
Mebendazole	95–100	95–100	45–100	96	44
Albendazole	33–95	67–100	10–77	40–100	17–95
Thiabendazole					89–100*
Pyrantel	37–88[†]	81–100	0–56	>90	
Levamisole	66–100	86–100	16–18		
Ivermectin[‡]	0–20	50–75	11–80	61–94	83–100
Piperazine		74–94		*c.* 90	

The hookworm data include the cure rates for *A. duodenale* and *N. americanus* collectively.

*Thiabendazole is used worldwide as the standard treatment for *S. stercoralis* infections.

[†]Pyrantel pamoate is particularly effective against *A. duodenale*.

[‡]Ivermectin is not yet registered for use against human GI nematodes, and has only recently been registered for use against *S. stercoralis* in France, Australia and the USA.

Sources: Freedman *et al.* (1989); Naquira *et al.* (1989); Albonico *et al.* (1999); Horton (2000); Burkhart & Burkhart (2005).

multiple doses, and generally show variable efficacy against the different species of human GI nematodes, with no drug being 100% effective against all nematode species (Table 2). For example, albendazole can reduce infections with *Ascaris*, hookworm and *Trichuris* in school children so that improvements in the growth, weight, physical fitness, cognition and nutrition of these children are evident (Stephenson *et al.* 1993). However, its efficacy varies against each species, in that the cure rates for *Ascaris*, hookworm and *Trichuris* have been reported as being 95%, 78% and 48%, respectively (Horton 2000). *Strongyloides stercoralis* is the least satisfactory human GI nematode to treat, as the efficacy of the most commonly used anthelmintics, such as mebendazole and pyrantel, is low and the most effective anthelmintic in this case is thiabendazole. However, side-effects with this drug are common, occurring in 50% of cases, and include nausea, dizziness, weakness, anorexia, vomiting and/or headache. Although albendazole and ivermectin are more effective with fewer adverse side-effects, and ivermectin has recently been registered for use in France, Australia and the USA, thiabendazole still remains the standard drug for treatment (Cook 1986; Stürchler 1987; Schneider & Rogers 1997).

Sanitation, hygiene and education

The transmission of all human GI nematodes, with the exception of *E. vermicularis*, is abolished when all human faeces are adequately and thoroughly disposed of in latrines because humans are the only host for these species and, as explained earlier, the only exit route for transmission stages is via human faeces. For all species, but especially for *E. vermicularis*, good personal hygiene and high levels of cleanliness are very important for the prevention of infection. The treatment of all family members simultaneously is also very important for the prevention of infection with *E. vermicularis* because the female worms of this species contaminate bed clothes with infective eggs while infected people sleep (Gonzalez & Javier de la Cabada 1987; Cook 1994; Kucik *et al.* 2004). Since humans are gregarious animals and mostly sleep in family groups, this transmission strategy is extremely effective, hence the cosmopolitan distribution of pinworms irrespective of climate or geographical location.

As mentioned earlier, dogs may act as reservoirs of important zoonotic parasites worldwide, a role that is of even more significance in areas of promiscuous defecation. In one area of North Eastern India, eggs of *T. trichiura* and *A. lumbricoides*, believed to be host-specific for humans, were found in the faeces of dogs, particularly those kept as pets. These eggs were found to be viable, suggesting that dogs can act as transmitters of infection with *Ascaris* or

Trichuris between humans under these conditions, probably by ingesting infected human faeces from which the eggs pass through the gut without hatching and are then passed out in dog faeces. Increasing the knowledge and awareness of the general population to parasitic zoonoses, particularly those from dogs, and educating communities on the importance of latrines, would greatly reduce the prevalence of GI nematodes (Traub *et al.* 2002, 2005). However, direct contact with dogs infected with *T. canis* is also important in parasite transmission to humans because the eggs may also be found on the coats of infected dogs (Wolfe & Wright 2003). Thus, improvements in general hygiene are crucial for a reduction in transmission of these parasites.

Together with improvements in health education and general hygiene and sanitation, better housing, sewage disposal, water supply and health care are required for sustainable helminth control. Under these circumstances, the strategic application of chemotherapeutic drugs will maintain reductions in worm burden, morbidity and transmission, particularly in endemic areas (Roos 1997). Improvements in sanitation and hygiene are important to reduce the risk of reinfection and, in combination with anthelmintic drugs, may improve the growth and development of millions of children (Albonico *et al.* 1999; Stephensen 1999). Effective sewage disposal will also prevent recurring GI nematode infections, and the use of footwear is an important preventive method against hookworm infections (Gilles 1968; Gonzalez & Javier de la Cabada 1987; Kucik *et al.* 2004). Health education also has a major role in these integrated control programmes to discourage geophagy, which is linked to infection intensity and prevalence of *A. lumbricoides*, *T. trichiura* and *T. canis* (Geissler *et al.* 1998; Glickman *et al.* 1999). As school children are most at risk of infection with these parasites, and have the highest infection intensity, control programmes involving school children should have the greatest effect on parasite transmission (Bundy *et al.* 1987, 1988). However, the greatest intensity of hookworm infection occurs in the older members of the community, and so control programmes against hookworms should involve this sector of the community for optimal effect.

Although treatment with the current anthelmintics can significantly reduce parasite burdens, chemotherapy alone is unlikely to prevent recurring infections with GI nematodes. Infections are best controlled by integrated strategies, involving the provision of clean water, better housing, improvements in sanitation and hygiene, health education and the general nutritional status, and the strategic use of combinations of chemotherapeutic anthelmintics. Using such integrated control programmes, strategic utilization of the current anthelmintic drugs will prolong their efficacy and delay the

onset of resistance (Schantz 1991; Coles 1995; Reynoldson *et al.* 1998; Albonico *et al.* 1999; Dossa *et al.* 2001).

Problems with current strategies to control GI nematode infections of humans

The control of parasitic nematode infections is made difficult by environmental, social and economic factors. Complete eradication of helminths is impossible without major changes to socio-economic conditions throughout the world, and without the associated financial investment required to provide these (Albonico *et al.* 1999). Therefore, an alternative strategy is to accept that worm infections cannot be eradicated and then to try to keep infections as low as possible through reduction of transmission of the parasite, thereby limiting both the number of people at risk of infection and the associated morbidity (Crompton 1999).

A major problem in the control of GI nematode infections is the development of resistance to the currently available anthelmintics. Resistance to the drugs in all three of the major anthelmintic classes used in livestock is widespread, particularly in Africa, Australia, New Zealand, Asia and South America (Waller 1997), and there is now a potential danger of the occurrence of resistance in helminths of humans. However, the treatment of humans with anthelmintics is less frequent than that of livestock and occurs as part of better control programmes, which should slow the appearance of resistance in human helminths (Geerts *et al.* 1997; Roos 1997; Geerts & Gryseels 2001; Horton 2003). Unfortunately, it is a case of when, not if, resistance develops. Currently, the benzimidazoles are the drugs which are the most frequently used to treat human GI nematode infections, but it is to these drugs that livestock nematodes have developed the most resistance (Coles 1995). Thus, the spread of resistance among GI nematodes of livestock to the available anthelmintics provides a crucial warning about the widespread use of anthelmintics to control human GI nematodes (Coles 1995; Geerts & Gryseels 2000). Indeed, there have been recent reports indicating reductions in efficacy and the possible development of resistance to anthelmintics in the human population. For instance, in southeast Mali, mebendazole and pyrantel have recently shown worryingly low efficacy against human infections with *N. americanus* (de Clercq *et al.* 1997; Sacko *et al.* 1999). Similarly, in the Kimberly region of North Western Australia, pyrantel lacks efficacy against *A. duodenale*, possibly through development of resistance as a result of frequent use of this drug (Reynoldson *et al.* 1997). On Pemba Island, Zanzibar, the efficacy of mebendazole against hookworms in school children appeared to have

fallen over a period of 5 years, during which time the children were regularly treated with mebendazole (egg reduction rate fell from 82.4% to 52.1%). This suggests the possibility of emergence of mebendazole-resistant hookworms on Pemba Island, and provides a crucial warning about the consistent use of anthelmintics as the sole means to control human GI nematode infections (Albonico *et al.* 1994, 2003).

The problem of the development of resistance is particularly worrying because there are few new drugs at the clinical trial stage of development. However, recent studies have identified some potential candidates. In Peru and Mexico, nitazoxanide, currently only available to treat protozoan infections, has been successfully used against ascariasis, trichuriasis and strongyloidiasis, with few adverse side-effects (Davila-Gutierrez *et al.* 2002; Ortiz *et al.* 2002). Unfortunately, there are also no vaccines available for the treatment of human GI nematode infections and there are unlikely to be any in the foreseeable future.

Strategies for delaying the onset of resistance, and making the most use of the available anthelmintics, include selective treatment of populations. By treating only a proportion of the affected community, usually those who are most likely to harbour heavy infections, or by treating at a time of the year when significant proportions of worms are in the host's external environment, the selection pressure on worms with resistant alleles is not as intense as it might otherwise have been. The worms that avoid treatment (either in non-treated individuals or in the environment as eggs or larvae) represent 'worms in refugia' with non-resistant genotypes. Thus, at the parasite population level, non-resistant worms survive episodes of treatment and, in this way, help to dilute the resistant alleles in the population and slow down the increase of resistance that would otherwise occur if all worms were subjected to the anthelmintic in question (Smith 1990; Smith *et al.* 1999; Coles 2002, 2005).

Other strategies for slowing the onset of resistance include the application of combinations of different anthelmintic drugs. For instance, pyrantel on its own has very poor efficacy against *T. trichiura*, but in the combination pyrantel–oxantel (oxantel being another group 2 drug), the egg reduction rate was greater than 80% (Albonico *et al.* 2002). Administering levamisole and mebendazole together was found to significantly increase the efficacy against *A. lumbricoides*, *T. trichiura* and hookworms compared with either drug alone (Albonico *et al.* 2003). It is likely that increased combination therapy will slow the development of resistance, particularly when combinations involving different drugs with distinct modes of action are used (Smith 1990; Coles 2006). Combination therapy is, therefore,

advantageous and should be used as the treatment of choice against soil-transmitted helminths.

Another form of combination therapy which could be used to control intestinal nematode infections is to combine treatment programmes for different diseases, such as malaria, schistosomiasis, intestinal helminthiasis and filariasis (Molyneux & Nantulya 2004), where drug combinations such as ivermectin and albendazole, or praziquantel and albendazole, are used to increase the spectrum of efficacy. Two early studies examined the effect of the combination of ivermectin and albendazole against the filarial nematode, *Wuchereria bancrofti*, and the intestinal helminths, *A. lumbricoides*, *T. trichiura* and hookworms. Both studies demonstrated that the combined treatment resulted in a significantly greater reduction in the prevalence and intensity of infection of all three intestinal helminths and *W. bancrofti*. Therefore, integrated control programmes for different diseases would probably substantially improve the health of infected populations, especially children (Addiss *et al.* 1997; Beach *et al.* 1999). However, these integrated control programmes are only in their early stages.

In the developing countries, one of the major problems in the control of GI nematode infections is the expense of anthelmintics in relation to family budgets even though, in some parts of the world, prices have fallen in recent years, and anthelmintics may cost only a fraction of the price charged in developed countries. Even so, cheaper, less efficient drugs, such as tetrachlorethylene, may be used or treatments may be shared between family members, so that each member receives less than the recommended dose (Cook 1986). The use of sub-optimal doses encourages the spread of parasite genotypes that have resistant alleles to anthelmintics (Smith 1990). A much greater expense in the control of human GI nematode infections, especially in the developing countries, is the provision of clean water supplies, better sanitation and good primary health care (Guyatt & Evans 1992).

Thus, it is clear that there are numerous problems associated with the control of GI nematode infections, and that novel methods are required.

The search for new anthelmintics

One alternative strategy may be the use of natural products that have anthelmintic properties. There has been a recent resurgence in the investigation of naturally occurring substances for their anthelmintic properties (for examples see Guarrera 1999; Waller *et al.* 2001; Githiori *et al.* 2003; Hördegen *et al.* 2003; Hounzangbe-Adote *et al.* 2005a,b), and this has been largely due to the presence of resistance to the current chemotherapeutic anthelmintics. The material under study is often plant-derived and this line of investigation has been encouraged by the fact that such plant extracts have been used traditionally by indigenous people, mainly in the tropics, against GI nematodes of both humans and livestock. To date, very few of these putative natural anthelmintics have been comprehensively assessed scientifically (e.g. Raj 1974; Desta 1995; Tandon *et al.* 1997; McGaw *et al.* 2000; Giday *et al.* 2003; Beloin *et al.* 2005). Perhaps the most encouraging data have been provided by a group of plant extracts rich in cysteine proteinase activity (Table 3; Stepek *et al.* 2004). These include wound-induced latices from species of fig, papaya and milkweed, and extracts from the fruit, stem and leaves of

Table 3 Known plant sources of cysteine proteinases

Plant source	Plant location	Enzyme	Source
Carica papaya (papaya)	Latex of plant, including unripe fruit	Papain	Balls (1937)
		Chymopapain	Jansen and Balls (1941)
		Caricain	Robinson (1975)
		Glycyl endopeptidase	Buttle *et al.* (1989)
Ananas comosus (pineapple)	Fruit	Fruit Bromelain	Chittenden (1894)
	Stem	Stem Bromelain	Heinicke and Gortner (1957)
		Ananain	Rowan *et al.* (1988)
		Comosain	Napper *et al.* (1994)
Ficus carica (fig)	Latex of plant, including unripe fruit	Ficin	Kramer and Whitaker (1964)
Ficus glabrata (fig)	Latex of plant, including unripe fruit	Ficin	Englund *et al.* (1968)
Actinidia chinensis (kiwi fruit)	Fruit	Actinidain	Brocklehurst *et al.* (1981)
Calotropis gigantea (madar plant)	Latex of plant	Calotropin	Abraham and Joshi (1979)
Calotropis procera	Latex of plant	Procerain	Dubey and Jagannadham (2003)
Asclepias syriaca (milkweed)	Latex of plant	Asclepain	Brockbank and Lynn (1979)

pineapple. For instance, *Ficus glabrata* latex is a well-known anthelmintic in Central and South America. Since the start of the last century, several other species of fig, such as *Ficus laurifolia*, have also been used against *Trichuris*, *Taenia*, *Ascaris* and *Enterobius*. In addition, papaya (*Carica papaya*) and pineapple (*Ananas comosus*) have been used to treat chickens, dogs, pigs and humans infected with intestinal parasites. However, use of these traditional treatments only occurred in areas where the plants were found, and their use quickly declined when the modern synthetic drugs were introduced (Gaughran 1976).

With the onset of resistance to the synthetic anthelmintics, interest in this area of research has now been rekindled, although satisfactory trials in humans that conform to contemporary standards are still largely lacking. In one rare example, in the Amazon basin, the crude latex of *F. glabrata* was administered orally for three consecutive days at 1 ml/kg. After treatment, there were reductions in egg output of *Ascaris* (85%), *Strongyloides* (72%), *Ancylostoma/Necator* (55%) and *Trichuris* (67%), almost matching the efficacy of the modern, synthetic anthelmintics used as positive controls. No adverse side-effects associated with the use of fig latex were noted (Hansson *et al.* 1986). Studies in livestock are almost equally as rare, although anecdotal stories suggest the successful use, as early as the 19th century, of the crude latex of *C. papaya* against ascarids, tapeworms, whipworms and hookworms (Berger & Asenjo 1940). In two well-documented trials, a single dose of the crude latex of *C. papaya* provided comparable anthelmintic efficacy to the currently available synthetic anthelmintics, with respect to reductions in the egg output and worm burden, in pigs infected with the roundworm, *Ascaris suum* (Satrija *et al.* 1994) and mice infected with *Heligmosomoides polygyrus*, one of the most common laboratory models for routine screening of potential drug candidates (Satrija *et al.* 1995).

Although the above studies describe anthelmintic efficacy of the extracts of papaya and fig, the mechanism of action by which these extracts produced their effects was not assessed. This is important because current drug legislation requires an understanding of the mode of action of candidate drugs before registration for use as alternative treatments against GI nematode infections in humans. *In vitro*, *F. laurifolia* latex (Robbins 1930) and *F. glabrata* latex (Hansson *et al.* 1986) acted rapidly against *A. suum*, causing the nematode cuticle to become wrinkled and flaccid, before developing small blisters, which later perforated releasing the internal structures, and causing the death of the nematode (Robbins 1930). Purified papain from papaya latex (Berger & Asenjo 1940) and bromelain from fresh pineapple juice (Berger & Asenjo 1939) also caused rapid ulceration of *A. suum*,

followed by digestion of the worm *in vitro*. These studies, taken together, indicated that the anthelmintic properties of pineapple juice and of the latex of *Ficus* species and of *C. papaya* had a similar mechanism of action, which differed to that of the currently available anthelmintics. Most of these plants are widely available and inexpensive in tropical and sub-tropical countries, the areas with highest infection intensity.

Proof that the active principles in these plant extracts were cysteine proteinases was provided by Stepek *et al.* (2005), using *H. polygyrus in vitro*. The plant extracts, or cysteine proteinases purified from them, only possessed anthelmintic activity if a reducing agent (cysteine) was present. These enzymes are well known to be active only in a reducing environment. In addition, the specific inactivator of the papain family of cysteine proteinases, L-*trans*-epoxysuccinyl-leucylamido-4-guanidino-butane (E-64), completely inhibited the anthelmintic activity. It was also demonstrated that this mechanism was specific to cysteine proteinases, in that it did not occur with aspartic or serine proteinases found in the mammalian alimentary canal. Digestion of the cuticle also occurred when the same enzymes were incubated *in vitro* with the rodent GI nematodes, *Trichuris muris* (Stepek *et al.* 2006), *Protospirura muricola* (G. Stepek *et al.*, personal communication) and *A. ceylanicum* (G. Stepek *et al.*, personal communication). The same mechanism of action was also observed *in vivo* against *H. polygyrus*, resulting in the expulsion of only the affected worms from the murine gastrointestinal tract (G. Stepek *et al.*, personal communication). Moreover, fresh latex from the fig plants, *Ficus carica* and *Ficus insipida*, also partially digested the cestode, *Rodentolepis* (*Vampirolepis*) *nana*, and the oxyurids, *Syphacia obvelata* and *Aspiculuris tetraptera* (de Amorin *et al.* 1999). The mechanism of action is, therefore, not restricted to one nematode species, but has the same detrimental effect on a range of GI nematodes and cestodes.

Potential problems of plant cysteine proteinases as anthelmintics

The cysteine proteinases from plants may seem to be a viable alternative to current anthelmintics, but there are some potential problems associated with these enzymes. It is well known that papain and its homologues are inactive at low pH. This is for two reasons, the first being due to the *p*Ka of the essential thiolate anion, which is normally around pH 4. This means that at pH values below 4 (such as in the stomach) the enzymes will be largely inactive. The second reason is that these enzymes suffer irreversible denaturation at low pH such that, following passage through the

stomach, the enzymes may be irreversibly inactivated even though the pH then rises to close to their pH optimum of 7–8. It has recently been suggested that these properties, as well as digestion by the stomach proteinase pepsin, may mean that the anthelmintic proteinases need to be protected against low pH to be effective as anthelmintics (Huet *et al.* 2006). All the successful *in vivo* trials published to date have utilized impure enzyme preparations, such as crude papaya latex. Our recent data demonstrated that, when delivered orally in the form of crude latex, sufficient enzyme activity survived passage through the entire gastrointestinal tract of mice to mediate a potent anthelmintic effect against a nematode species residing in the lower alimentary tract (Stepek *et al.* 2006). Possibly the presence of the proteinaceous latex material is sufficient to locally buffer the stomach contents against acidity (which would also inactivate pepsin), thus allowing the enzymes to traverse this otherwise hostile environment. We have found that administration of pure papain to mice infected with *H. polygyrus* resulted in much lower efficacy than administration of the same amount of cysteine proteinase in the form of crude latex (G. Stepek *et al.*, unpublished data). It is to be expected that improved formulations and methods of delivery will increase efficacy of the pure enzymes in the future, especially as Hale (2004) has recently shown that when bromelain (from the pineapple, *Ananas comosus*) was administered with antacid, it retained its activity throughout the intestinal tract of mice.

Although the toxicities of the purified enzymes appear to be quite low (chymopapain is already a licensed drug for the treatment of intervertebral disc disease, and many of the plant cysteine proteinases have been used as meat tenderisers and in other aspects of food preparation), problems associated with allergy may occur. However, these usually arise following intake through the airways, as is commonly the case with immunoglobulin E (IgE)-mediated allergy (Soto-Mera *et al.* 2000; Nelde *et al.* 2001), and serious allergic reactions following oral ingestion would be expected to be extremely rare. However, Hale (2004) found anti-bromelain antibodies in the serum of mice following prolonged (18 weeks) daily oral administration of the enzyme. Bromelain is known to erode some of the cell surface markers essential for inflammatory responses, affect the production of cytokine secretion, and generally show anti-inflammatory activity when administered *in vivo* (Hale & Haynes 1992; Hale *et al.* 2002). However, the intestinal mucosa has a rapid turnover (2–3 days) in rodents, and normal function would be rapidly restored after either a single oral treatment or a week of daily treatments. Other causes of toxicity may be associated with oral administration of some crude latex preparations. For instance, doses of 10 ml/kg/day for 3 days of *F. carica* or *F. insipida* latex produced fatally high toxicity levels in mice (de Amorin *et al.* 1999).

As with other control strategies, reinfection is certain to occur if improvements in hygiene and sanitation standards are not made, and so a combination of anthelmintic treatment and better hygiene is required. Thus, if improvements in sanitation and hygiene are not made and sustained, the use of plant cysteine proteinases as anthelmintics may not provide much benefit. On the other hand, plant cysteine proteinases, as novel anthelmintics, will only add to the range of tools and associated strategies that aim to maintain low prevalence of infection and low morbidity rather than eradication. In this context, since these enzymes occur naturally in tropical plants, they offer scope for local production and may spawn novel agricultural enterprises for developing countries. This, in turn, may be facilitated by the development of new varieties of the source plants that contain more active enzymes at greater concentrations, through classic selection programmes or transgenesis once the genes responsible have been identified. At the individual level, they offer cheap and regular supplies of medicines for the treatment of nematode infections of impoverished people in the developing countries.

Conclusions

At the present time, control of GI nematode infections of humans is not totally adequate, despite the existence of chemical anthelmintics of varying effectiveness. The inevitability of the development of resistance to the current anthelmintics will complicate the future control of GI nematode infections in humans unless new drugs are discovered, or the existing anthelmintics are used strategically to avoid the occurrence of resistance. New anthelmintic drugs, with mechanisms of action that differ from those that are currently available, are desperately required for the control of GI nematodes. In the absence of any new candidates in chemical drug development, the cysteine proteinases found naturally in plants, such as papaya, pineapple and fig, represent a viable option. These treatments would be relatively inexpensive, are likely to be without serious adverse reactions and have been used by indigenous people of tropical countries for centuries. Improved methods of delivery will be expected to bring further advances in terms of efficacy and safety, and it is imperative that the plant cysteine proteinases are tested to modern pharmaceutical standards and that their potential benefits are assessed in controlled studies. Such work should be undertaken with all possible haste, as the protection given by the current chemical anthelmintics may soon be limited.

References

Abraham K.I. & Joshi P.N. (1979) Studies on proteinases from *Calotropis gigantea* latex. I: Purification and some properties of two proteinases containing carbohydrate. *Biochim. Biophys. Acta* **568**, 111–119.

Addiss D.G., Beach M.J., Streit T.G. *et al.* (1997) Randomised placebo-controlled comparison of ivermectin and albendazole alone and in combination for *Wuchereria bancrofti* microfilaraemia in Haitian children. *Lancet* **350**, 480–484.

Albonico M., Smith P.G., Hall A., Chwaya H.M., Alawi K.S., Savioli L. (1994) A randomised controlled trial comparing mebendazole and albendazole against *Ascaris*, *Trichuris* and hookworm infections. *Trans. R. Soc. Trop. Med. Hyg.* **88**, 585–589.

Albonico M., Stoltzfus R.J., Savioli L. *et al.* (1998) Epidemiological evidence for a differential effect of hookworm species, *Ancylostoma duodenale* or *Necator americanus*, on iron status of children. *Int. J. Epidem.* **27**, 530–537.

Albonico M., Crompton D.W.T., Savioli L. (1999) Control strategies for human intestinal nematode infections. *Adv. Parasitol.* **42**, 277–341.

Albonico M., Bickle Q., Haji H.J. *et al.* (2002) Evaluation of the efficacy of pyrantel-oxantel for the treatment of soil-transmitted nematode infections. *Trans. R. Soc. Trop. Med. Hyg.* **96**, 685–690.

Albonico M., Bickle Q., Ramsan M., Montresor A., Savioli L., Taylor M. (2003) Efficacy of mebendazole and levamisole alone or in combination against intestinal nematode infections after repeated targeted mebendazole treatment in Zanzibar. *Bull. WHO* **81**, 343–352.

de Amorin A., Borba H.R., Carauta J.P.P., Lopes D., Kaplan M.A.C. (1999) Anthelmintic activity of the latex of *Ficus* species. *J. Ethnopharm.* **64**, 255–258.

Ayash-Rashkovsky M., Weisman Z., Diveley J., Moss R.B., Bentwich Z., Borkow G. (2002) Generation of Th1 immune responses to inactivated, gp120-depleted HIV-1 in mice with a dominant Th2 biased immune profile via immunostimulatory oligonucleotides – relevance to AIDS vaccines in developing countries. *Vaccine* **20**, 2684–2692.

Baboolal S. & Rawlins S.C. (2002) Seroprevalence of toxocariasis in schoolchildren in Trinidad. *Trans. R. Soc. Trop. Med. Hyg.* **96**, 139–143.

Balls A.K. (1937) Crystalline papain. *Science* **86**, 379.

Beach M.J., Streit T.G., Addiss D.G., Prospere R., Roberts J.M., Lammie P.J. (1999) Assessment of combined ivermectin and albendazole for treatment of intestinal helminth and *Wuchereria bancrofti* infections in Haitian schoolchildren. *Am. J. Trop. Med. Hyg.* **60**, 479–486.

Beloin N., Gbeassor M., Akpagana K. *et al.* (2005) Ethnomedicinal uses of *Momordica charantia* (Cucurbitaceae) in Togo and relation to its phytochemistry and biological activity. *J. Ethnopharm.* **96**, 49–55.

Bentwich Z., Kalinkovich A., Weisman Z. (1995) Immune activation is a dominant factor in the pathogenesis of African Aids. *Immunol. Today* **16**, 187–191.

Bentwich Z., Kalinkovich A., Weisman Z., Borkow G., Beyers N., Beyers A.D. (1999) Can eradication of helminthic infections change the face of Aids and tuberculosis? *Immunol. Today* **20**, 485–487.

Berger J. & Asenjo C.F. (1939) Anthelmintic activity of fresh pineapple juice. *Science* **90**, 299–300.

Berger J. & Asenjo C.F. (1940) Anthelmintic activity of crystalline papain. *Science* **91**, 387–388.

Booth M. & Bundy D.A.P. (1992) Comparative prevalences of *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm infections and the prospects for combined control. *Parasitology* **105**, 151–157.

Booth M., Bundy D.A.P., Albonico M., Chwaya H.M., Alawi K.S., Savioli L. (1998) Associations among multiple geohelminth species infections in schoolchildren from Pemba Island. *Parasitology* **116**, 85–93.

Borkow G. & Bentwich Z. (2000) Eradication of helminthic infections may be essential for successful vaccination against HIV and tuberculosis. *Bull. WHO* **78**, 1368–1369.

Boycott A.E. (1911) Ankylostoma infection. *Lancet* **1**, 717–721.

Boycott A.E. & Haldane J.S. (1903) An outbreak of ankylostomiasis in England. *J. Hyg.* **3**, 95–136.

Brockbank W.J. & Lynn K.R. (1979) Purification and preliminary characterisation of two asclepains from the latex of *Asclepias syriaca* L. (milkweed). *Biochim. Biophys Acta* **578**, 13–22.

Brocklehurst K., Baines B.S., Malthouse J.P.G. (1981) Differences in the interactions of the catalytic groups of the active centres of actinidin and papain. Rapid purification of fully active actinidin by covalent chromatography and characterization of its active centre by use of two-protonic-state reactivity probes. *Biochem. J.* **197**, 739–746.

Bundy D.A.P., Cooper E.S., Thompson D.E., Didier J.M., Simmons I. (1987) Epidemiology and population dynamics of *Ascaris lumbricoides* and *Trichuris trichiura* infection in the same community. *Trans. R. Soc. Trop. Med. Hyg.* **81**, 987–993.

Bundy D.A.P., Kan S.P., Rose R. (1988) Age-related prevalence, intensity and frequency distribution of gastrointestinal helminth infection in urban slum children from Kuala Lumpur, Malaysia. *Trans. R. Soc. Trop. Med. Hyg.* **82**, 289–294.

Bundy D.A.P., Chan M.S., Savioli L. (1995) Hookworm infection in pregnancy. *Trans. R. Soc. Trop. Med. Hyg.* **89**, 521–522.

Burkhart C.N. & Burkhart C.G. (2005) Assessment of frequency, transmission, and genitourinary complications of enterobiasis (pinworms). *Int. J. Dermatol.* **44**, 837–840.

Buttle D.J., Kembhavi A.A., Sharp S.L., Shute R.E., Rich D.H., Barrett A.J. (1989) Affinity purification of the novel cysteine proteinase papaya proteinase IV and papain from papaya latex. *Biochem. J.* **261**, 469–476.

Bwibo N.O. (1971) Clinical significance of strongyloides in African children. *J. Trop. Med. Hyg.* **74**, 79–81.

Chan M.-S. (1997) The global burden of intestinal nematode infections – fifty years on. *Parasitol. Today* **13**, 438–443.

Chittenden R.H. (1894) On the proteolytic action of bromelin, the ferment of pineapple juice. *J. Physiol.* **15**, 249–310.

de Clercq D., Sacko M., Behnke J., Gilbert F., Dorny P., Vercruysse J. (1997) Failure of mebendazole in treatment of human hookworm infections in the southern region of Mali. *Am. J. Trop. Med. Hyg.* **57**, 25–30.

Clinch C.R. & Stephens M.B. (2000) Case description of ascariasis. *Arch. Fam. Med.* **9**, 1193–1194.

Coles G.C. (1995) Chemotherapy of human nematodes: learning from the problems in sheep. *J. Roy. Soc. Med.* **88**, 649P–651P.

Coles G.C. (2002) Cattle nematodes resistant to anthelmintics: why so few cases? *Vet. Res.* **33**, 481–489.

Coles G.C. (2005) Anthelmintic resistance – looking to the future: a UK perspective. *Res. Vet. Sci.* **78**, 99–108.

Coles G.C. (2006) Anthelmintic resistance and anthelmintic combinations. *Vet. Rec.* (in press).

Conway D.J., Hall A., Anwar K.S., Rahman M.L., Bundy D.A.P. (1995) Household aggregation of *Strongyloides stercoralis* infection in Bangladesh. *Trans. R. Soc. Trop. Med. Hyg.* **89**, 258–261.

Cook G.C. (1986) The clinical significance of gastrointestinal helminths – a review. *Trans. R. Soc. Trop. Med. Hyg.* **80**, 675–685.

Cook G.C. (1994) *Enterobius vermicularis* infections. *Gut* **35**, 1159–1162.

Coop R.L. & Kyriazakis I. (2001) Influence of host nutrition on the development and consequences of nematode parasitism in ruminants. *Trends. Parasitol.* **17**, 325–330.

Crompton D.W.T. (1986) Nutritional aspects of infection. *Trans. R. Soc. Trop. Med. Hyg.* **80**, 697–705.

Crompton D.W.T. (1999) How much human helminthiasis is there in the world? *J. Parasitol.* **85**, 397–403.

Davila-Gutierrez C.E., Vasquez C., Trujillo-Hernandez B., Huerta M. (2002) Nitazoxanide compared with quinfamide and mebendazole in the treatment of helminthic infections and intestinal protozoa in children. *Am. J. Trop. Med. Hyg.* **66**, 251–254.

Desta B. (1995) Ethiopian traditional herbal drugs. Part I: studies on the toxicity and therapeutic activity of local taenicidal medications. *J. Ethnopharm.* **45**, 27–33.

Dossa R.A.M., Ategbo E.A.D., de Koning F.L.H.A., Van Raaij J.M.A., Hautvast J.G.A.J. (2001) Impact of iron supplementation and deworming on growth performance in preschool Beninese children. *Eur. J. Clin. Nutr.* **55**, 223–228.

Dreyfuss M.L., Stoltzfus R.J., Shrestha J.B. *et al.* (2000) Hookworms, malaria and vitamin A deficiency contribute to anaemia and iron deficiency among pregnant women in the plains of Nepal. *J. Nutr.* **130**, 2527–2536.

Dubey V.K., Jagannadham M.V. (2003) Procerain, a stable cysteine protease from the latex of *Calotropis procera*. *Phytochemistry* **62**, 1057–1071.

Elliott A.M., Kyosiimire J., Quigley M.A. *et al.* (2003) Eosinophilia and progression to active tuberculosis in HIV-1-infected Ugandans. *Trans. R. Soc. Trop. Med. Hyg.* **97**, 477–480.

Englund P.T., King T.P., Craig L.C., Walti A. (1968) Studies on ficin I: its isolation and characterisation. *Biochemistry* **7**, 163–175.

Fisher D., McCarry F., Currie B. (1993) Strongyloidiasis in the northern territory: under-recognised and under-treated?. *Med. J. Aust.* **159**, 88–90.

Foster W.D. (1965) *A History of Parasitology*. E. & S. Livingstone Ltd, London.

Freedman D.O., Zierdt W.S., Lujan A., Nutman T.B. (1989) The efficacy of ivermectin in the chemotherapy of gastrointestinal helminthiasis in humans. *J. Infect. Dis.* **159**, 1151–1153.

Gaughran E.R.L. (1976) Ficin: history and present status. *Quart. J. Crude Drug Res.* **14**, 1–21.

Geerts S. & Gryseels B. (2000) Drug resistance in human helminths: current situation and lessons from livestock. *Clin. Microbiol. Rev.* **13**, 207–222.

Geerts S. & Gryseels B. (2001) Anthelmintic resistance in human helminths: a review. *Trop. Med. Int. Health* **6**, 915–921.

Geerts S. Coles G.C., Gryseels B. (1997) Anthelmintic resistance in human helminths: learning from the problems with worm control in livestock. *Parasitol. Today* **13**, 149–151.

Geissler P.W., Mwaniki D., Thiong'o F., Friis H. (1998) Geophagy as a risk factor for geohelminth infections: a longitudinal study of Kenyan primary schoolchildren. *Trans. R. Soc. Trop. Med. Hyg.* **92**, 7–11.

Giday M., Asfaw Z., Elmqvist T., Woldu Z. (2003) An ethnobotanical study of medicinal plants used by the Zay people in Ethiopia. *J. Ethnopharm.* **85**, 43–52.

Gilgen D., Mascie-Taylor C.G.N., Rosetta L. (2001) Intestinal helminth infections, anaemia and labour productivity of female tea pluckers in Bangladesh. *Trop. Med. Int. Health* **6**, 449–457.

Gilles H.M. (1968) Gastrointestinal helminthiasis. *BMJ* **2**, 475–477.

Githiori J.B., Höglund J., Waller P.J., Baker R.L. (2003) Evaluation of anthelmintic properties of extracts from some plants used as livestock dewormers by pastoralist and smallholder farmers in Kenya against *Heligmosomoides polygyrus* infections in mice. *Vet. Parasitol.* **118**, 215–226.

Glickman L.T., Camara A.O., Glickman N.W., McCabe G.P. (1999) Nematode intestinal parasites of children in rural Guinea, Africa: prevalence and relationship to geophagia. *Int. J. Epidem.* **28**, 169–174.

Gonzalez S. & Javier de la Cabada F. (1987) Parasitic infections of the colon and rectum. *Baillière's Clin. Gastroenterol.* **1**, 447–467.

Guarrera P.M. (1999) Traditional antihelmintic, antiparasitic and repellent uses of plants in Central Italy. *J. Ethnopharmacol.* **68**, 183–192.

Guyatt H. (2000) Do intestinal nematodes affect productivity in adulthood? *Parasitol. Today* **16**, 153–158.

Guyatt H.L. & Evans D. (1992) Economic considerations for helminth control. *Parasitol. Today* **8**, 397–402.

Hale L.P. (2004) Proteolytic activity and immunogenicity of oral bromelain within the gastrointestinal tract of mice. *Int. Immunopharmacol.* **4**, 255–264.

Hale L.P. & Haynes B.F. (1992) Bromelain treatment of human T cells removes CD44, CD45RA, E2/MIC2, CD6, CD7, CD8 and Lue 8/LAM1 surface molecules and markedly enhances CD2-mediated T cell activation. *J. Immunol.* **149**, 3809–3816.

Hale L.P., Greer P.K., Sempowski G.D. (2002) Bromelain treatment alters leukocyte expression of cell surface molecules involved in cellular adhesion and activation. *Clin. Immunol.* **104**, 183–190.

Hansson A., Veliz G., Naquira C., Amren M., Arroyo M., Arevalo G. (1986) Preclinical and clinical studies with latex from *Ficus glabrata* HBK, a traditional intestinal anthelmintic in the Amazonian region. *J. Ethnopharm.* **17**, 105–138.

Hartleb M. & Januszewski K. (2001) Severe hepatic involvement in visceral larva migrans. *Eur. J. Gastroenterol. Hepatol.* **13**, 1245–1249.

Heinicke R.M. & Gortner W.A. (1957) Stem bromelain – a new protease preparation from pineapple plants. *Econ. Bot.* **11**, 225–234.

Hördegen P., Hertzberg H., Heilmann J., Langhans W., Maurer V. (2003) The anthelmintic efficacy of five plant products against gastrointestinal trichostrongylids in artificially infected lambs. *Vet. Parasitol.* **117**, 51–60.

Horton J. (2000) Albendazole: a review of anthelmintic efficacy and safety in humans. *Parasitology* **121**, S113–S132.

Horton J. (2003) Human gastrointestinal helminth infections: are they now neglected diseases? *Trends Parasitol.* **19**, 527–531.

Hounzangbe-Adote S., Fouraste I., Moutairou K., Hoste H. (2005a) *In vitro* effects of four tropical plants on the activity and development of the parasitic nematode, *Trichostrongylus colubriformis*. *J. Helminthol.* **79**, 29–33.

Hounzangbe-Adote M.S., Paolini V., Fouraste I., Moutairou K., Hoste H. (2005b) *In vitro* effects of four tropical plants on three life-cycle stages of the parasitic nematode, *Haemonchus contortus*. *Res. Vet. Sci.* **78**, 155–160.

Huet J., Looze Y., Bartik K., Raussens V., Wintjens R., Boussard P. (2006) Structural characterization of the papaya cysteine proteinases at low pH. *Biochem. Biophys. Res. Commun.* **341**, 620–626.

Jain A.K., Agarwal S.K., EL-Sadr W. (1994) *Streptococcus bovis* bacteremia and meningitis associated with *Strongyloides stercoralis* colitis in a patient infected with Human Immunodeficiency Virus. *Clin. Infect. Dis.* **18**, 253–254.

Jansen E.F. & Balls A.K. (1941) Chymopapain: a new crystalline proteinase from papaya latex. *J. Biol. Chem.* **137**, 459–460.

Kappus K.D., Lundgren R.G., Juranek D.D., Roberts J.M., Spencer H.C. (1994) Intestinal parasitism in the United States: update on a continuing problem. *Am. J. Trop. Med. Hyg.* **50**, 705–713.

Keiser P.B. & Nutman T.B. (2004) *Strongyloides stercoralis* in the immunocompromised population. *Clin. Microbiol. Rev.* **17**, 208–217.

Killewo J.Z.J., Cairncross S., Smet J.E.M., Maikwano L.F., Van Asten H. (1991) Patterns of hookworm and *Ascaris* infection in Dar es Salaam. *Acta Trop.* **48**, 247–249.

Kramer D.E. & Whitaker J.R. (1964) *Ficus* enzymes II: properties of the proteolytic enzymes from the latex of *Ficus carica* variety Kadota. *J. Biol. Chem.* **239**, 2178–2183.

Kucik C.J., Martin G.L., Sortor B.V. (2004) Common intestinal parasites. *Am. Fam. Phys.* **69**, 1161–1168.

Levav M., Mirsky A.F., Schantz P.M., Castro S., Cruz M.E. (1995) Parasitic infection in malnourished school children: effects on behaviour and EEG. *Parasitology* **110**, 103–111.

McGaw L.J., Jäger A.K., Van Staden J. (2000) Antibacterial, anthelmintic and anti-amoebic activity in South African medicinal plants. *J. Ethnopharm.* **72**, 247–263.

Milder J.E., Walzer P.D., Kilgore G., Rutherford I., Klein M. (1981) Clinical features of Strongyloides stercoralis infection in an endemic area of the United States. *Gastroenterology* **80**, 1481–1488.

Molyneux D.H. & Nantulya V.M. (2004) Linking disease control programmes in rural Africa: a pro-poor strategy to reach Abuja targets and millennium development goals. *BMJ* **328**, 1129–1132.

Napper A.D., Bennett S.P., Borowski M. *et al.* (1994) Purification and characterization of multiple forms of the pineapple-stem-derived cysteine proteinases ananain and comosain. *Biochem. J.* **301**, 727–735.

Naquira C., Jimenez G., Guerra J.G. *et al.* (1989) Ivermectin for human strongyloidiasis and other intestinal helminths. *Am. J. Trop. Med. Hyg.* **40**, 304–309.

Nelde A., Teufel M., Hahn C. *et al.* (2001) The impact of the route and frequency of antigen exposure on the IgE response in allergy. *Int. Arch. Allergy Immunol.* **124**, 461–469.

Nokes C., Grantham-McGregor S.M., Sawyer A.W., Cooper E.S., Robinson B.A., Bundy D.A.P. (1992a) Moderate to heavy infections of *Trichuris trichiura* affect cognitive function in Jamaican school children. *Parasitology* **104**, 539–547.

Nokes C., Grantham-McGregor S.M., Sawyer A.W., Cooper E.S., Bundy D.A.P. (1992b) Parasitic helminth infection and cognitive function in school children. *Proc. R. Soc. Lond. B* **247**, 77–81.

Ortiz J.J., Chegne N.L., Gargala G., Favennec L. (2002) Comparative clinical studies of nitazoxanide, albendazole and praziquantel in the treatment of ascariasis, trichuriasis and hymenolepiasis in children from Peru. *Trans. R. Soc. Trop. Med. Hyg.* **96**, 193–196.

Othieno C., Hirsch C.S., Hamilton B.D., Wilkinson K., Ellner J.J., Toossi Z. (1999) Interaction of *Mycobacterium tuberculosis*-induced transforming growth factor β1 and interleukin-10. *Infect. Immun.* **67**, 5730–5735.

Prociv P. & Croese J. (1996) Human enteric infection with *Ancylostoma caninum*: hookworms reappraised in the light of a 'new' zoonosis. *Acta Trop.* **62**, 23–44.

Raj R.K. (1974) Screening of some indigenous plants for anthelmintic action against human *Ascaris lumbricoides*. *Indian J. Phys. Pharmacol.* **18**, 129–131.

Rang H.P., Dale M.M., Ritter J.M., Moore P.K. (2003) Chapter 49: Anthelmintic drugs. In: *Pharmacology*, 5th edn. pp. 687–692 (eds. H.P. Rang, M.M. Dale, J.M. Ritter, P.K. Moore). London: Churchill Livingston.

Reynoldson J.A., Behnke J.M., Pallant L.J. *et al.* (1997) Failure of pyrantel in treatment of human hookworm infections (*Ancylostoma duodenale*) in the Kimberly region of North West Australia. *Acta Trop.* **68**, 301–312.

Reynoldson J.A., Behnke J.M., Gracey M. *et al.* (1998) Efficacy of albendazole against *Giardia* and hookworm in a remote Aboriginal community in the north of Western Australia. *Acta Trop.* **71**, 27–44.

Robbins B.H. (1930) A proteolytic enzyme in ficin, the anthelmintic principle of leche de higueron. *J. Biol. Chem.* **87**, 251–257.

Robinson G.W. (1975) Isolation and characterization of papaya peptidase A from commercial chymopapain. *Biochemistry* **14**, 3695–3700.

Roos M.H. (1997) The role of drugs in the control of parasitic nematode infections: must we do without? *Parasitology* **114**, S137–S144.

Rowan A.D., Buttle D.J., Barrett A.J. (1988) Ananain: a novel cysteine proteinase found in pineapple stem. *Arch. Biochem. Biophys.* **267**, 262–270.

Sacko M., de Clercq D., Behnke J.M., Gilbert F.S., Dorny P., Vercruysse J. (1999) Comparison of the efficacy of mebendazole, albendazole and pyrantel in treatment of human hookworm infections in the southern region of Mali, West Africa. *Trans. R. Soc. Trop. Med. Hyg.* **93**, 195–203.

Satrija F., Nansen P., Bjørn H., Murtini S., He S. (1994) Effect of papaya latex against *Ascaris suum* in naturally infected pigs. *J. Helminthol.* **68**, 343–346.

Satrija F., Nansen P., Murtini S., He S. (1995) Anthelmintic activity of papaya latex against patent *Heligmosomoides polygyrus* infections in mice. *J. Ethnopharm.* **48**, 161–164.

Schantz P.M. (1991) Parasitic zoonoses in perspective. *Int. J. Parasitol.* **21**, 161–170.

Schneider J.H. & Rogers A.I. (1997) Strongyloidiasis: the protean parasitic infection. *Postgrad. Med.* **102**, 177–184.

Simeon D.T., Grantham-McGregor S.M., Callender J.E., Wong M.S. (1995) Treatment of *Trichuris trichiura* infections improves growth, spelling scores and school attendance in some children. *J. Nutr.* **125**, 1875–1883.

Smith G. (1990) A mathematical model for the evolution of anthelmintic resistance in a direct life-cycle nematode parasite. *Int. J. Parasitol.* **20**, 913–921.

Smith G., Grenfell B.T., Isham V., Cornell S. (1999) Anthelmintic resistance revisited: under-dosing, chemoprophylactic strategies, and mating probabilities. *Int. J. Parasitol.* **29**, 77–91.

Soto-Mera M.T., López-Rico M.R., Filgueira J.F., Villamil E., Cidrás R. (2000) Occupational allergy to papain. *Allergy* **55**, 983–984.

Stepek G., Behnke J.M., Buttle D.J., Duce I.R. (2004) Natural plant cysteine proteinases as anthelmintics? *Trends Parasitol.* **20**, 322–327.

Stepek G., Buttle D.J., Duce I.R., Lowe A., Behnke J.M. (2005) Assessment of the anthelmintic effect of natural plant cysteine proteinases against the gastrointestinal nematode, *Heligmosomoides polygyrus*, in vitro. *Parasitology* **130**, 203–211.

Stepek G., Lowe A.E., Buttle D.J., Duce I.R., Behnke J.M. (2006) In vitro and in vivo anthelmintic efficacy of plant cysteine proteinases against the rodent gastrointestinal nematode, *Trichuris muris*. *Parasitology* **132**, 681–689.

Stephensen C.B. (1999) Burden of infection on growth failure. *J. Nutr.* **129**, 534S–538S.

Stephenson L.S., Latham M.C., Adams E.J., Kinoti S.N., Pertet A. (1993) Physical fitness, growth and appetite of Kenyan school boys with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* infections are improved four months after a single dose of albendazole. *J. Nutr.* **123**, 1036–1046.

Stephenson L.S., Latham M.C., Ottesen E.A. (2000) Malnutrition and parasitic helminth infections. *Parasitology* **121**, S23–S38.

Stürchler D. (1987) Parasitic diseases of the small intestinal tract. *Baillière's Clin. Gastroenterol.* **1**, 397–424.

Tandon V., Pal P., Roy B., Rao H.S.P., Reddy K.S. (1997) *In vitro* anthelmintic activity of root-tuber extract of *Flemingia vestita*, an indigenous plant in Shillong, India. *Parasitol. Res.* **83**, 492–498.

Traub R.J., Robertson I.D., Irwin P., Mencke N., Thompson R.C.A. (2002) The role of dogs in transmission of gastrointestinal parasites in a remote tea-growing community in northeastern India. *Am. J. Trop. Med. Hyg.* **67**, 539–545.

Traub R.J., Robertson I.D., Irwin P.J., Mencke N., Thompson R.C.A.A. (2005) Canine gastrointestinal parasitic zoonoses in India. *Trends Parasitol.* **21**, 42–48.

Tsai H.-C., Lee S.S.-J., Liu Y.-C. *et al.* (2002) Clinical manifestations of strongyloidiasis in southern Taiwan. *J. Microbiol. Immunol. Infect.* **35**, 29–36.

Velasquez C.C. & Cabrera B.C. (1968) *Ancylostoma ceylanicum* (Looss, 1911) in a Filipino woman. *J. Parasitol.* **54**, 430–431.

Wakelin D. (1996a) Chapter 1: Parasites and parasitism. In: *Immunity to Parasites: How Parasitic Infections are Controlled*. 2nd edn. pp. 4–8 (ed. D. Wakelin). Cambridge: Cambridge University Press.

Wakelin D. (1996b) Chapter 7: Gastrointestinal Nematodes. In: *In Immunity to Parasites: How Parasitic Infections are Controlled*, 2nd edn. pp. 122–126 (ed. D. Wakelin). Cambridge: Cambridge University Press.

Waller P.J. (1997) Anthelmintic resistance. *Vet. Parasitol.* **72**, 391–412.

Waller P.J., Bernes G., Thamsborg S.M. *et al.* (2001) Plants as de-worming agents of livestock in the Nordic countries: historical perspective, popular beliefs and prospects for the future. *Acta Vet. Scand.* **42**, 31–44.

Whitfield P.J. (1993) Chapter 2: Parasitic Helminths. In: *Modern Parasitology*, 2nd edn. pp. 24–52 (ed. F.E.G. Cox). Oxford: Blackwell Scientific Publications.

Wolfe A. & Wright I.P. (2003) Human toxocariasis and direct contact with dogs. *Vet. Rec.* **152**, 419–422.